FINAL REPORT FOR GRANT NsG-517/23-04-002

TITLE: The effects of weightlessness and other stresses associated with flight in space on pathogenicity and immunity.

CONTENTS:

Summary:

The objective of the research supported by the grant was to design and test the feasibility of an experiment to determine the effects of space flight on pathogenicity and immunity. To meet this objective the following steps were completed, and a discussion of each is given in more detail in another section of the report.

1. A mouse chamber suitable for housing and feeding five mice for two weeks with one servicing was designed for use with germfree or conventional mice. Although not tested, it is believed that the chamber would be suitable for use in space flight under reduced gravity and with considerable vibration.
2. Plasmodium berghei infections in three strains of mice were characterized to determine the validity of using the infection for studying immunity and pathogenesis in mice during flight. It is believed that controlled infections of P. berghei can be used to produce the desired conditions to study phagocytosis and erythropoiesis in animals while in flight.
3. Mice infected with Plasmodium berghei lose there ability to maintain normal body temperature and hypothermic mice consume considerably less oxygen than uninfected mice.
4. By controlling the infection by treatment, the degree of anemia can be controlled. Following reduction of the infection, marked erythropoietic activity occurs.
   If such an experiment were conducted on mice in conditions of reduced gravity, the effects on erythropoietic activity could be studied.
5. Radioiron was used to estimate erythropoietic activity in infected mice. Although of much basic interest, it is not likely that this method will be of value for studies in flight experiments since sequential sampling or large numbers of mice will be required.
6. Phagocytic activity by cells in the liver, spleen and bone marrow could be demonstrated by the use of radioiron. This procedure is reliable with small numbers of mice and without sequential sampling, and it is a good possibility for flight experiments.

Mouse Chamber:

The chamber is constructed of an outer stainless steel cylinder (Figure 1) which supports an inner stainless steel screen cylinder. One end of the inner cylinder is closed by the tube feeders which are 3/4" stainless steel tubes closed at one end and welded together in four fields. The opposite end of the cylinder is closed by the central plate of the suction cap which screws on to the outer cylinder joining the air passage to the suction line.

A filter cap closes one end with a central plate holding the filter against the closed ends of the feeder tubes. The outer flange of the filter cap holds the edge of the filter in place.

To use the mouse chamber for housing germfree mice, the assembled chamber is autoclaved with the filter in place and the suction end closed with mylar film. The sterile chamber is transferred into a germfree supply isolator, the suction cap is removed allowing free access to the inner cylinder for addition of diet and mice. The unit is closed and can then be held in the supply isolator until other chambers are supplied or immediately taken out of the isolator through a lock system. It is not necessary to apply suction on the chamber for several hours; diffusion of air is sufficient.

Outside the isolator, the serviced chamber should be attached to a suction line to provide air flow sufficient to provide O_2 remove CO_2, water vapor and other gasses. The rate of flow may be varied according to the size and number of animals. Back contamination is prevented by a germicidal trap.

The diet consists of a defined synthetic liquid diet (Nitrico-NBC) diluted 1:1 in 2% agar. This diet provides water and nutritional requirements for growing mice. In our work, no weight gain differences between mice feed conventional mouse diet with water separate and the agar-water-liquid diet could be found when feed two weeks to mice starting at 20 grams. Mice in the chamber feed by climbing into the feeder tubes. Only occasionally did the mice take food from the tubes.

Wastes drop through the screen and moisture is absorbed by an absorbant paper liner taped to the outer cylinder. The flow of air assists in drying the paper. It is believed that under conditions of weightlessness, the air flow will carry wastes away from the filter and into the germicidal trap.

Variations in the dimensions may be made easily to accomodate more or fewer mice; or for longer or shorter periods without servicing. Portions of the feeders may be covered by a disc which can be rotated by a motor in the center of the feeder unit. This would allow an alternation in diet during flight to provide a diet with a drug or radioisotope during portions of the flight.

The center opening in the feeder area may be used for a motor or for instrumentation. If germfree animals are used with instrumentation, dry sterilization and suitable seals will be required. A light may be desired for biorhythms experiments and this could be provided in the central opening.

The use of germfree animals would be of considerable value. The diet can be supplied without the addition of antimicrobials, wastes could be collected for analysis without the effects of bacterial decomposition, gas analysis would be more significant, infections which frequently result under moist and contaminable conditions would not be a problem, etc.

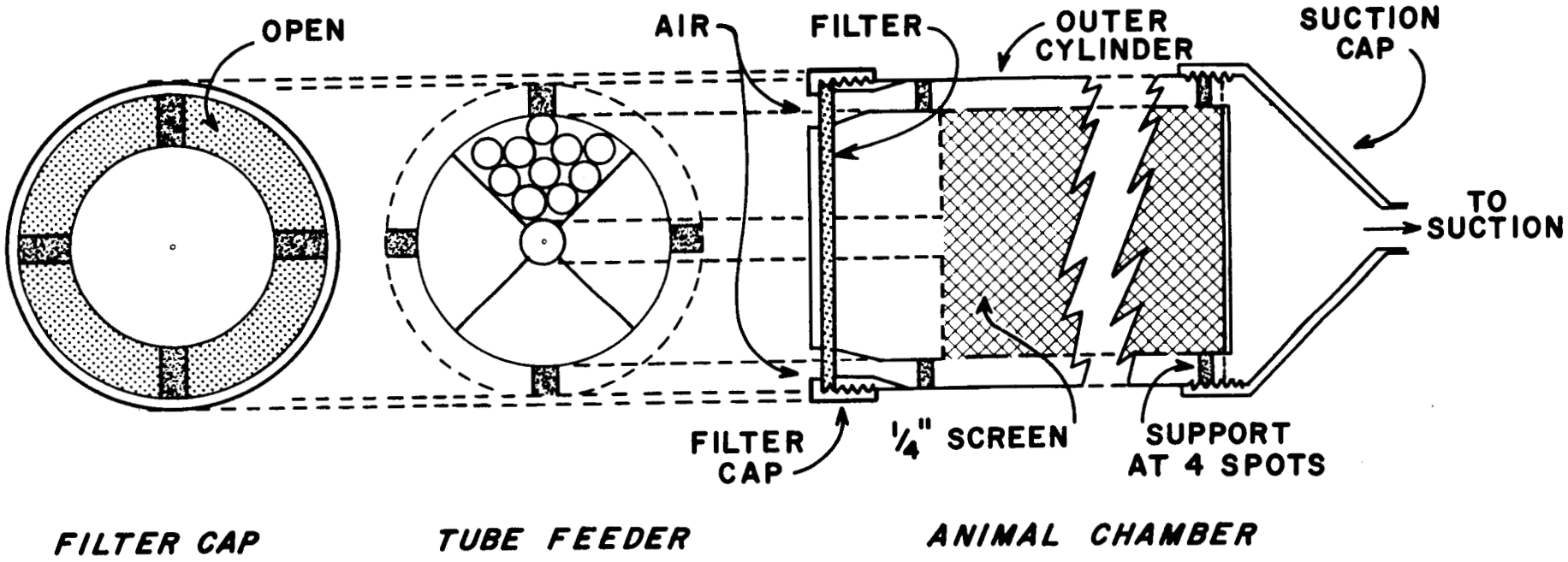
OPEN
AIR
FILTER
OUTER CYLINDER
SUCTION CAP
TO SUCTION
FILTER CAP
¼" SCREEN
SUPPORT AT 4 SPOTS
FILTER CAP
TUBE FEEDER
ANIMAL CHAMBER

FIGURE 1

The Course of Infection:

The parasitemia and red cell curves illustrated in Figure 2 are observed in essentially every Plasmodium berghei infection in Swiss Webster and CF-1 strains of mice when the inculum is approximately 1 x 10^6 parasites and administered intraperitoneally. The only variation in the parasitemia which occurred and is of importance is the time of death; varying from 10-14 days in 20 g mice and 16-20 days in 30 g mice.

An experiment designed to reduce the variation in death time was carried out as follows:

One million parasites were injected as precisely as possible into each of 50 mice. Blood films were made 48 hours later for the selection of 25 mice having 20-30 parasites in each oil immersion field; mice with more or less parasites were discarded. On the 5th day of infection, 12 mice were selected from the 25 previously selected based on the number of reticulocytes per field.

It was believed that the first selection would tend to equalize the parasites actually reproducing in the mice and the second selection would take into account variations in host response. Following this selection procedure in two groups of mice, the death times varied from 16-20 days in 30 g mice.

If reduced gravity and other factors associated with flight in space alter the course of P. berghei infections, the time of death should reflect the change. With the variation as great as 4 days, numerous animals would be required to identify the change as being significant. Since it is unrealistic to use large numbers of mice, other parameters were looked for which might indicate the effects of space flight on immunity and pathogenesis.

The change in the parasitemia curve on the 6th day of infection is possibly an expression of host defenses, but an unsuccessful attempt. It would be most interesting to know why the protective mechanisms fail.

Changes in Haptoglobin:

Absolute values for serum haptoglobin have not been determined. In our studies Jacobson's method was used. In this method the relative amounts of haptoglobin activity in infected and uninfected were determined and indicated as the rate of peroxidasic activity which results when haptoglobin is complexed with hemoglobin and the complex mixed with H_2O_2 and guanine.

It was expected that the amount of haptoglobin would be reduced in infected mice as a result of combination with free hemoglobin expected to occur with the destruction of red cells by the parasite. This does occur and by the 7th day of infection very little haptoglobin can be demonstrated in infected mice while uninfected mice continue to demonstrate normal levels.

It was observed that during the later stages of the infection (8th-15 days) after haptoglobins are at an exceedingly low level, no free hemoglobin is found in the serum and hemoglobinuria rarely occurs. Thus, an explanation must be found for the fate of hemoglobin during the infection. It is possible that the reduction in hemoglobin is almost entirely accounted for by the parasite's metabolism and that little free hemoglobin occurs outside of the red cells.

Mouse Body Temperature:

It was originally hoped that a temperature sensing device would be available to record the changes in body temperature during the course of infection and during flight. The device was not available; therefore rectal

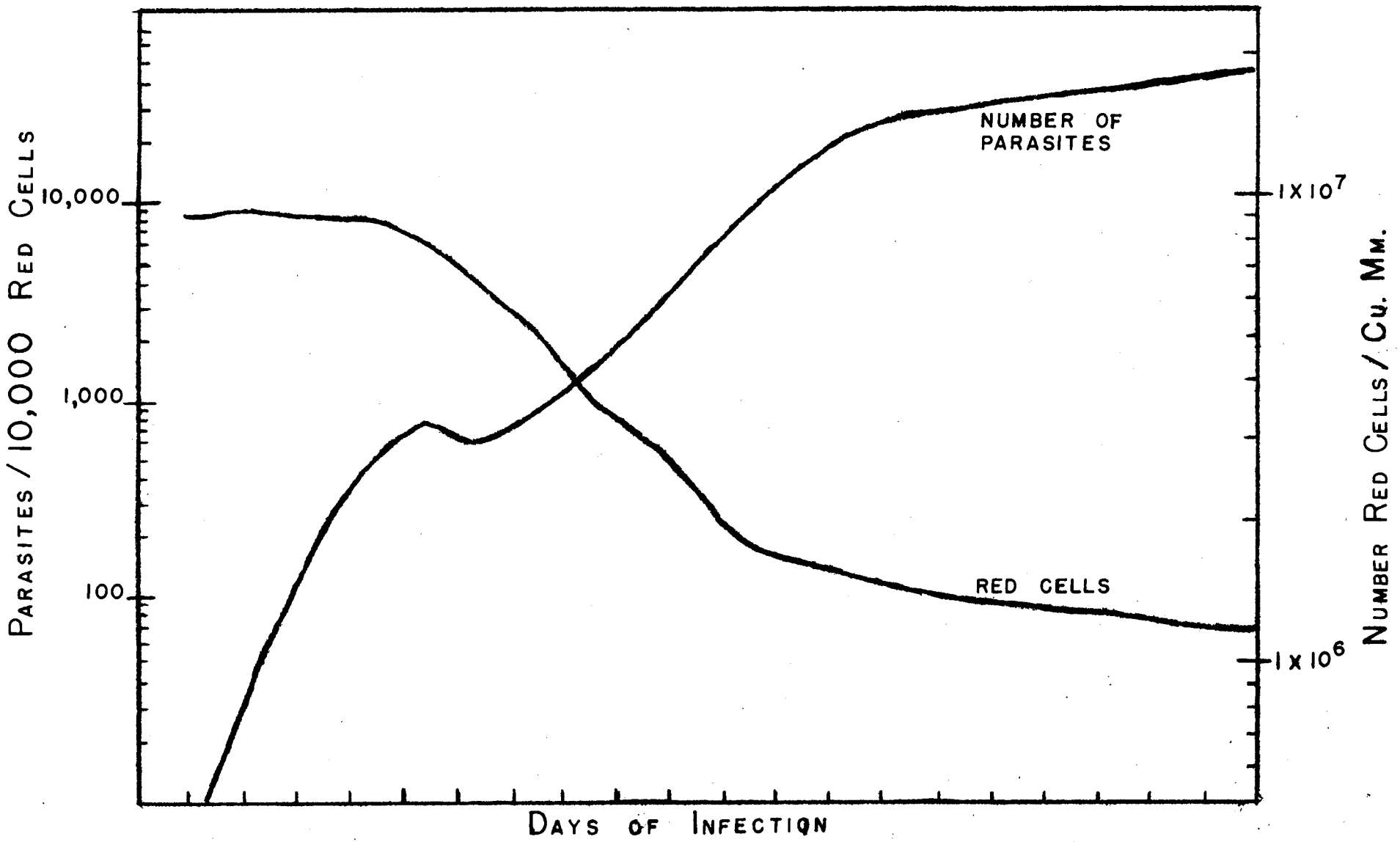
PARASITES / 10,000 RED CELLS
10,000
1,000
100
NUMBER OF PARASITES
RED CELLS
1 X 10^7
1 X 10^6
NUMBER RED CELLS / CU. MM.
DAYS OF INFECTION

FIGURE 2

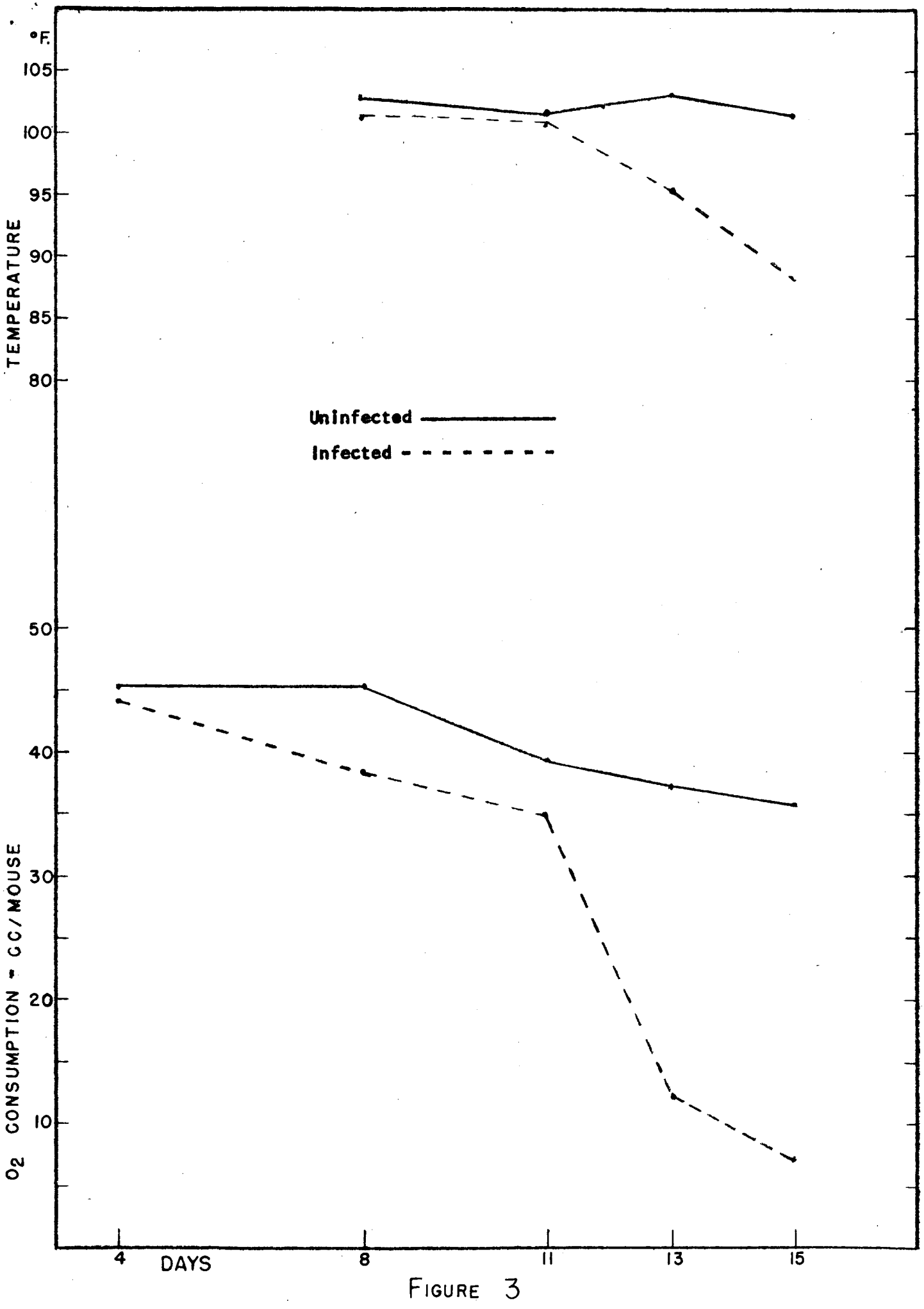
°F.
105
100
95
90
85
80
TEMPERATURE
Uninfected
Infected
50
40
30
20
10
O2 CONSUMPTION - CC/MOUSE
4
DAYS
8
11
13
15

FIGURE 3

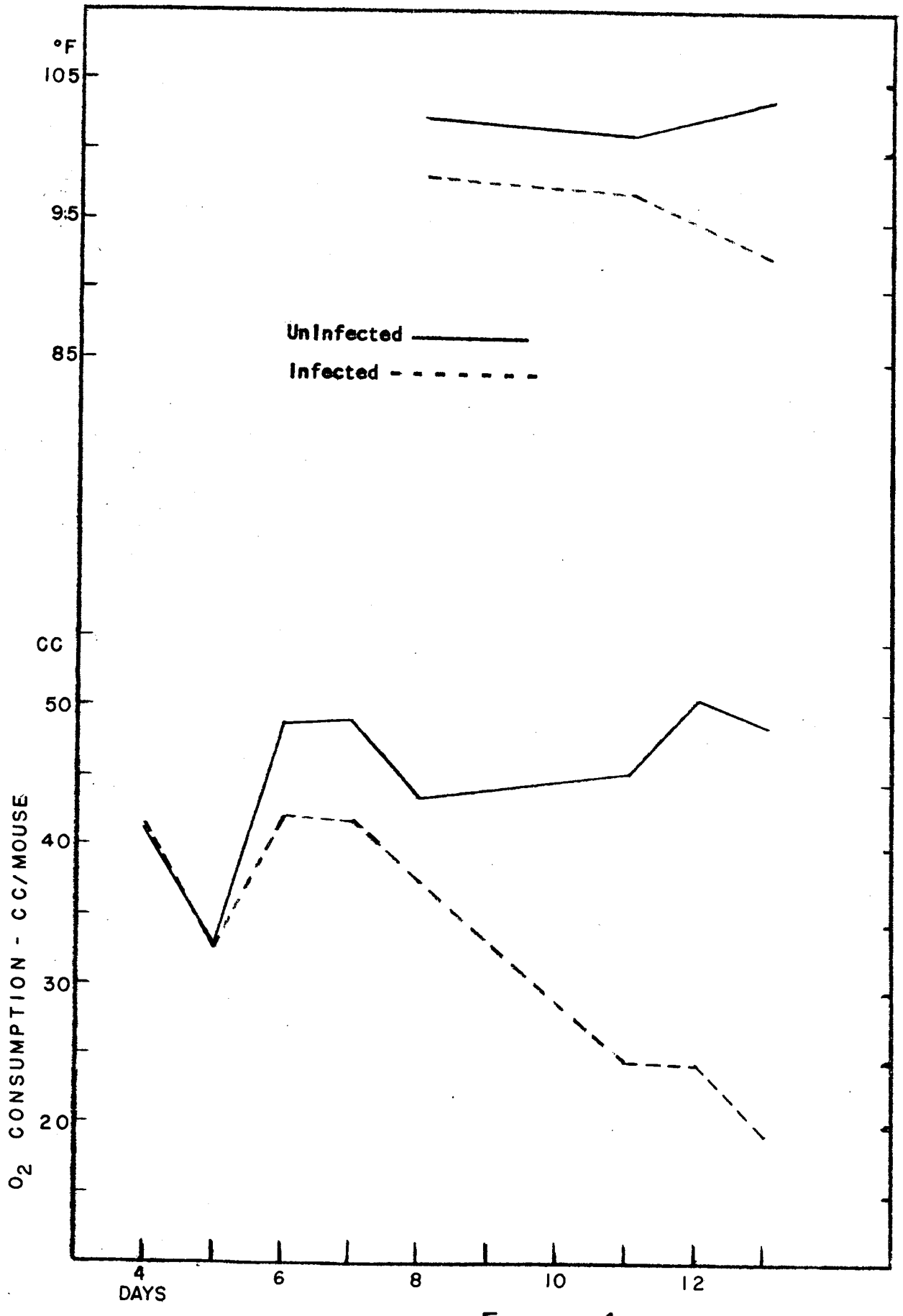
°F
105
95
85
Uninfected
Infected
cc
50
40
30
20
O_2 CONSUMPTION - CC/MOUSE
4
DAYS
6
8
10
12

FIGURE 4

temperatures were obtained at intervals by inserting thermistor probes into the rectum ½ to 3/4 inch. Figures 3 and 4 show the characteristic drop in body temperature which consistantly occurs in infected mice held at room temperature of 20-24°C. The mouse is unable to maintain normal body temperature after the 11th day of infection, and it is at this time the number of red cells are near the lowest for each individual. When hematocrit values drop to 25 %, both body temperature and O_2 consumption decrease. This correlation suggests that the failure to regulate body temperature in infected mice may be hypoxic hypothermia. The effect of the infection on liver function may also be of importance in temperature control.

Oxygen Consumption;

Oxygen consumption by uninfected mice and by mice during the course of infection are summarized in Figures 3 and 4. (Data in Tables). In Figure 3 it is easy to see that the drop in body temperature and O_2 consumption occur together, and this is at the time hematocrit values are around 25%.

Figures 5, 6 and 7 illustrate data presented in attached tables for individual mice. The O_2 consumption in all cases was determined by a differential manometer system attached to two chambers submerged in a waterbath for temperature control. The two chambers were essentially identical, but one contained the test mouse. Carbon dioxide was absorbed by KOH and water vapor was absorbed by Dririte. Most reliable values were obtained at the neutral temperature of 30°C; the O_2 consumption of normal mice being higher at both 37°C and 20°C than at 30°C. It is obvious from these data that O_2 consumption is greatly reduced in infected mice. The reduction could not be duplicated in mice by reducing food consumption or by phlebotomy to produce hematocrit values as low as 25%.

O_2 consumption by infected mice expressed per gram body weight rather than per mouse is approximately the same. Table 1 presents data to show this. When diet is restricted for uninfected mice to produce a loss in weight comparable to that which occurs in infected mice, the O_2 consumption remains at a level higher than for infected mice (Table 2). It is concluded that the decreased O_2 consumption by infected mice is not a function of decreased body tissue.

Clearance of Fe-59 from the Plasma:

In an attempt to better understand the effects of *P. berghei* infections on erythropoiesis, Iron-59 as ferrous citrate was injected IP into infected and uninfected mice and individuals killed at ½, 1 3/2, 2, 16, 24, 48 and 72 hour intervals. In all over 200 mice were used with 5 mice for each point for infected mice and 3 mice for each point for uninfected mice.

Figure 8 illustrates data obtained on mice injected with Fe-59 on the 6th day of infection. The percent of injected dose Fe-59 (%ID) in the plasma is not significantly different between infected and uninfected mice at any time. It would have been expected that mice with anemia produced by the infection would be actively producing red cells to compensate, and thus clear Fe-59 from the plasma more rapidly than uninfected mice. That this did not occur suggests that erythropoiesis is not occurring in infected mice at an increased rate.

The Fe-59 removed from the plasma of uninfected appears in the red cells, at 24 hours where it remains at a rather constant level. (Figure 9) In contrast the Fe-59 which first appears in the red cells of infected mice at 24 hours rapidly decreases. This is expected since during the course of infection, histiocytes actively phagocytize infected (and possibly uninfected)

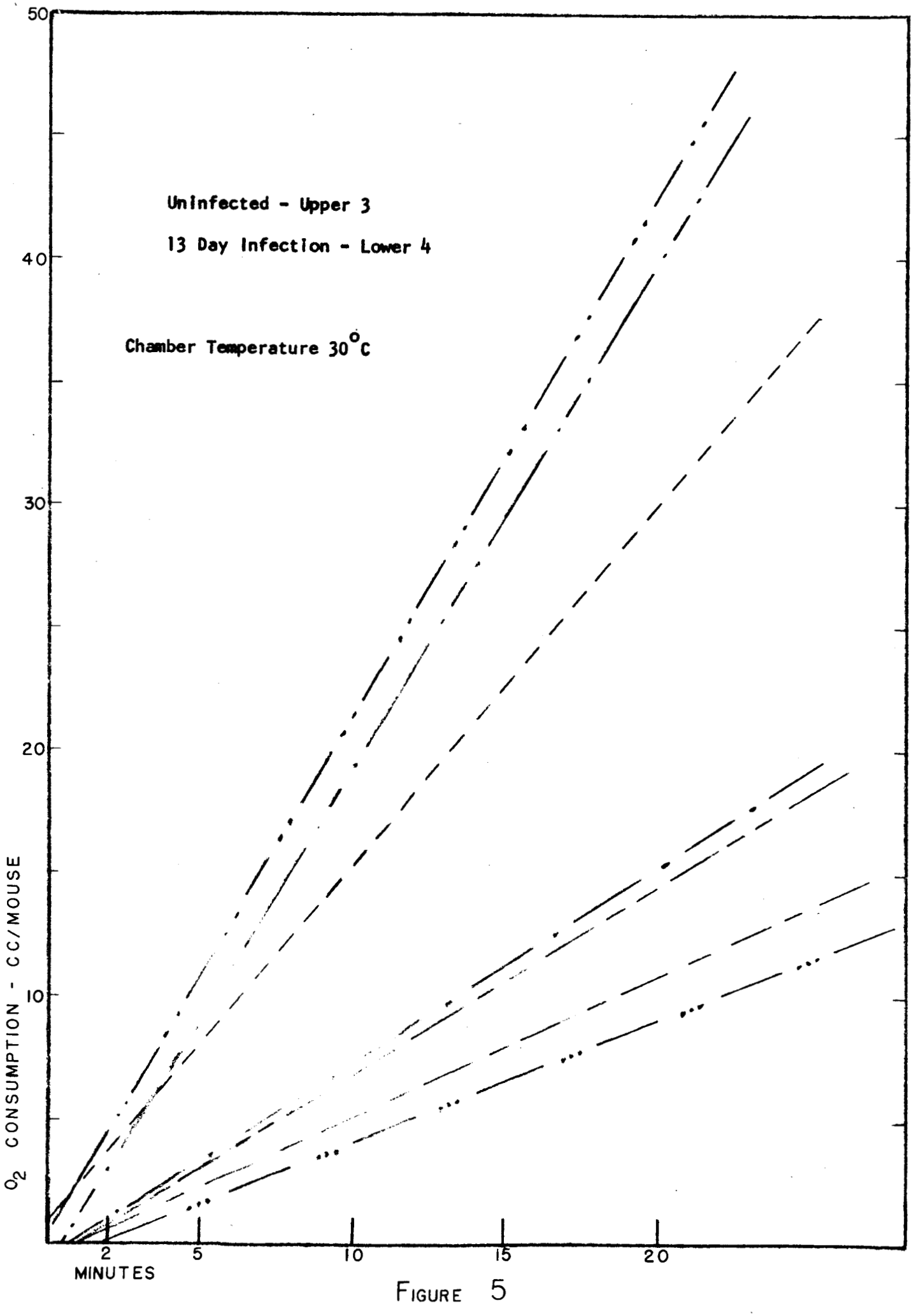
Uninfected - Upper 3
13 Day Infection - Lower 4
Chamber Temperature 30°C
50
40
30
20
10
O_2 CONSUMPTION - CC/MOUSE
2
5
10
15
20
MINUTES

FIGURE 5

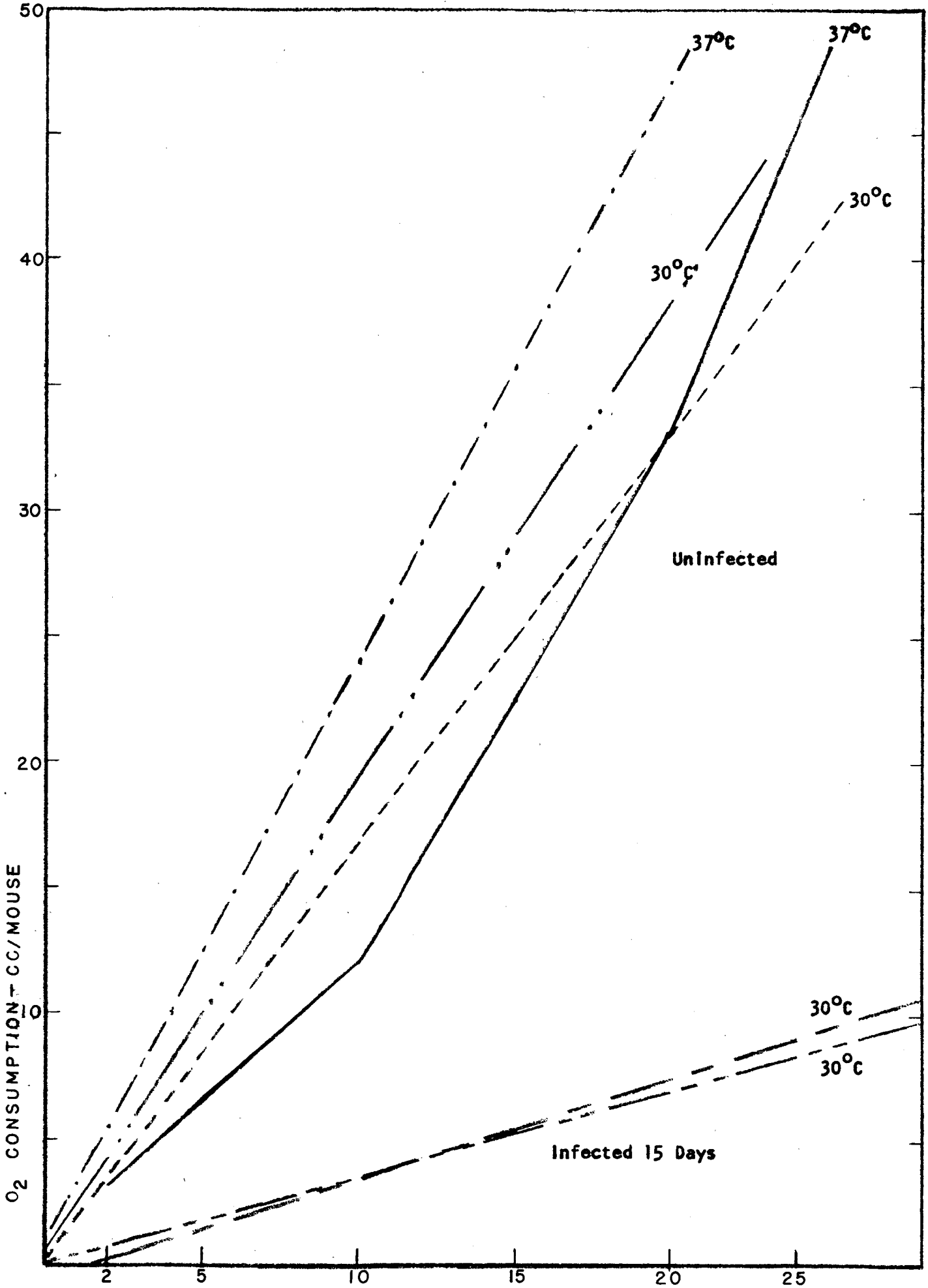
50
40
30
20
10
37°C
37°C
30°C
30°C
Uninfected
30°C
30°C
Infected 15 Days
O2 CONSUMPTION - CC/MOUSE
2
5
10
15
20
25
MINUTES

FIGURE 6

DATA FOR FIGURES 4 AND 5

O_2 Consumption cc/mouse/20 min

Days after Infecting	0	4	5	6	7	8	11	12	13	14
Date	5/7	5/11	5/12	5/13	5/14	5/15	5/18	5/19	5/20	5/21
INFECTED										
1		5600	2350	4050	4600	3975	2025	1500	1925	2175
2		3975	1925	4825	4900	3925	3250	4150	2525	3950
3		2875	2750	2775	2325	2375	Died	--	--	--
4		4100	4900	5000	4750	3700	2755	2725	1550	2925
5		4200	4400	4350	4250	4625	1750	1400	1750	1400
Mean		4150	3265	4200	4165	3720	2450	2443.7	1937.5	2612.5
CC.		41.5	32.7	42.0	41.7	37.2	24.5	24.4	19.4	26.1
UNINFECTED										
1		5700	2700	5025	5000	5000	4950	5525	5400	5900
2		2625	2650	4875	4575	3475	3900	4750	4600	6000
3		4050	4500	4725	5125	4550	4675	4900	4650	5525
Mean		4125	3283.3	4875	4900	4341.6	4508.3	5058.3	4883.3	5808.3
CC.		41.3	32.8	48.8	49.0	43.4	45.1	50.6	48.8	58.1
Chamber Temperature		30°C	30°C	30°C	30°C	30°C	30°C	30°C	30°C	20°C

DATA FOR FIGURES 4 AND 5

WEIGHT (Grams)

Days After Infecting	0	4	5	6	7	8	9&10	11	12	13	14
Date	5/7	5/11	5/12	5/13	5/14	5/15		5/18	5/19	5/20	5/21
INFECTED											
1	28	30			30			25		23.5	
2	28.5	32			33			33		30.5	
3	29	27.5			25			died--		--	
4	29.5	30.5			31			26.5		23.5	
5	30	33			33			28		24	
Mean	29	30.6			30.4			28.1		25.4	
UNINFECTED											
1	27	34			34.5			35.5		36	
2	28	29.5			30			30		31	
3	32	28.5			28			29		29.5	
Mean	29	30.7			30.8			31.5		32.2	

MOUSE TEMPERATURE

Room Temperature						80.5		81.8		--	
INFECTED											
1						97		97		93	
2						98.5		97.5		89.5	
3						98		--		--	
4						97.2		97		92	
5						99.2		96		94	
Mean						98.0		96.9		92.1	
UNINFECTED											
1						99.5		99		103	
2						103		100.5		103.5	
3						*104		*103.5		*104	

*Just after removing from O_2 chamber

Mean						102.2		101.0		103.5	

HEMATOCRIT (%)

INFECTED											
1		28		28					14	7	18
2		43		36					30	20	31
3		27		21					--	--	--
4		33		28					21	17	16
5		34		33					12	10	12
UNINFECTED											
1		44		49					49	49	
2		46		48					44	50	
3		43		46					44	36	

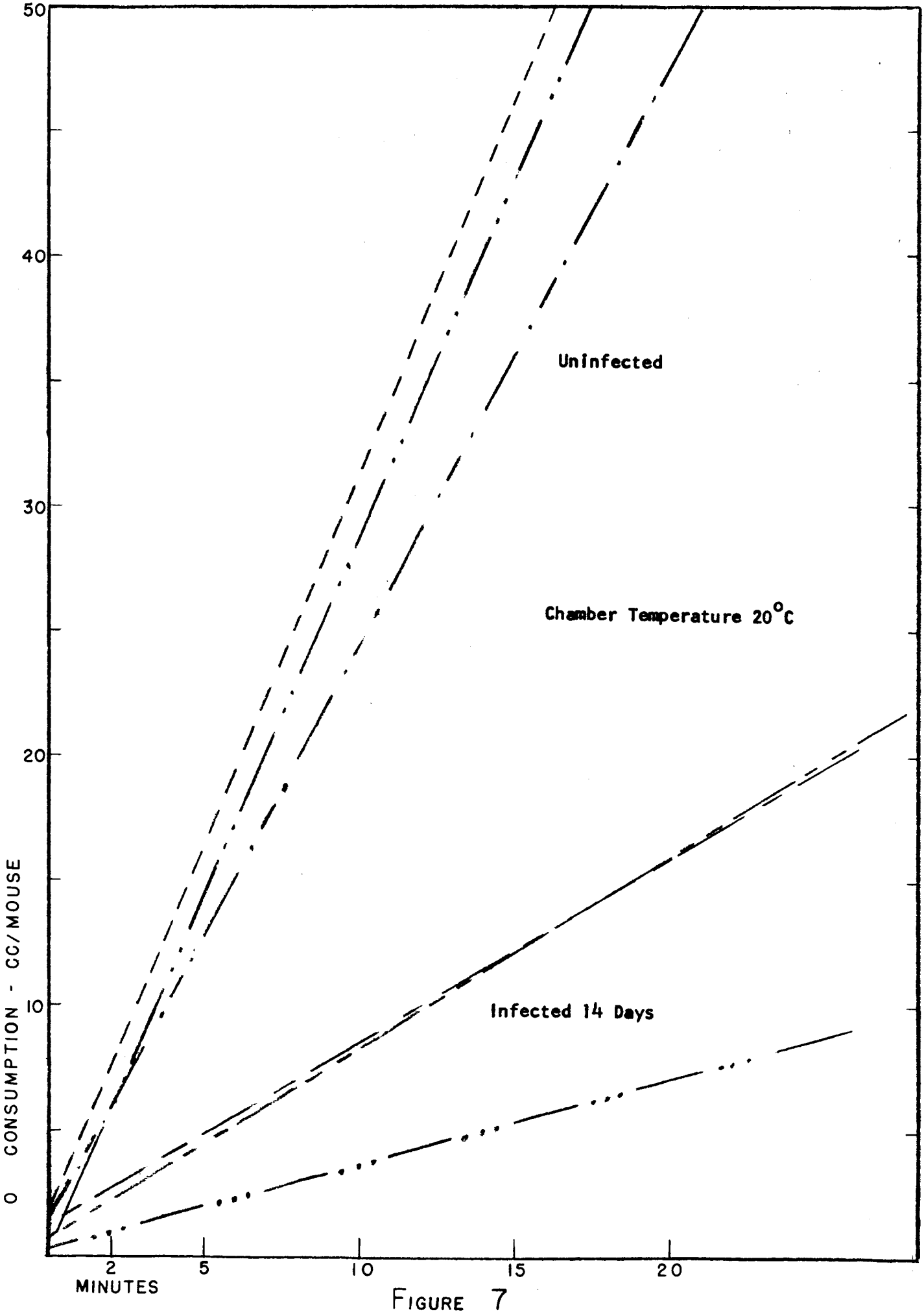
50
40
30
20
10
0
O CONSUMPTION - CC/MOUSE
Uninfected
Chamber Temperature 20°C
Infected 14 Days
2
5
10
15
20
MINUTES

FIGURE 7

DATA FOR FIGURES 3, 6 AND 7

WEIGHT (Grams)

Days After Infecting	0	4	8	11	13	14	15
Date	5/28	6/1	6/5	6/8	6/10	6/11	6/12
INFECTED							
1		33	33	28.5	--	--	--
2	31	33.5	32	29	25	--	--
3	39	37.5	36	33.5	28	26	24.5
4		38.5	37	33.5	--	--	--
5		40	39	35	30.5	28	26.5
7		30		24	22	20.5	--
Mean							
UNINFECTED							
1		38	38	37	38	38.5	40
2	27	32.5	35	32.5	30.5	32.5	33
3	38	35.5	35	32.5	32	32	
Mean							

MOUSE TEMPERATURE

INFECTED					
1	[1] 101.0	[1] 101.5	[1] --	[1] --	[1] --
2	102.5	100.0	96	--	--
3	101.0	100.8	95.7	81.2	89.5
4	101.0	101.0	--	--	--
5	101.2	101.2	96.5	83	87
7		[2] 91.5	93.2	75.2	[4] --
Mean	101.3	100.9	95.4	[3] 79.8	[4] 88.3
UNINFECTED					
1	104.0	100.7	102.5	100.8	101.5
2	102.8	101.5	102.2	102.2	101.2
3	101.5	102.5	104	96.2	
Mean	102.8	101.6	102.9	[3] 99.7	[4] 101.4

[1] All temps just after removing from chamber
[2] Not in jar - No 0_2 done on #7 for 6/8 - Not included in Mean
[3] After 0_2 run at 20°
[4] After 0_2 run at 30°, no temps taken after 37° run

HEMATOCRIT (%)

INFECTED			
1	40	27	
2	35	23	
3	49	35	
4	25	22	
5	38	27	
UNINFECTED			
1	52	46	36
2	44	*-	28
3	44	*-	

*Not taken - tails chewed up

DATA FOR FIGURES 3, 6 AND 7

O_2 Consumption cc/mouse/20 min.

Days After Infecting	0	4	8	11	13	14	15	15
Date	5/28	6/1	6/5	6/8	6/10	6/11	6/12	6/12
Chamber Temperature		30°C	30°C	30°C	30°C	20°C	30°C	37°C
INFECTED								
1		5200	3750	3425	--	--	--	--
2		4275	3900	3200	1525	--	--	--
3		4475	3800	3725	1075	1600	750	--
4		3575	3800	3400	--	--	--	--
5		4575	3925	3700	1450	1600	700	--
7					900	700		
Mean		4420	3835	3490	1237.5	1300	725	--
CC		44.2	38.4	34.9	12.4	13.0	7.3	
UNINFECTED								
1		5400	4325	3450	3000	6050	3325	4700
2		4475	4450	3975	4250	5700	3850	3325
3		3750	4850	4325	4000	4750		
Mean		4541.6	4541.6	3916.6	3750	5500	3582	4012
CC		45.4	45.4	39.2	37.5	55.0	35.9	40.1

TABLE I

Oxygen Consumption by Infected and Uninfected Mice Per Gram Body Weight. Diet ad libitum.

Days After Infection	4		5	6	7		8	11		12	13	
MOUSE INFECTED	WEIGHT	$cc0_2/g/hr$	$cc0_2/g/hr$	$cc0_2/g/hr$	WEIGHT	$cc0_2/g/hr$	$cc0_2/g/hr$	WEIGHT	$cc0_2/g/hr$	$cc0_2/g/hr$	WEIGHT	$cc0_2/g/hr$
1	30	5.60	2.35	4.05	30	4.60	3.98	25	2.44	1.80	23.5	2.46
2	32	3.73	1.81	4.53	33	4.45	3.58	33	2.95	3.78	30.5	2.49
3	27.5	3.14	3.00	3.03	25	2.80	2.85	--	--	--	--	--
4	30.5	4.03	4.82	4.92	31	4.60	3.58	26.5	3.15	3.10	2.35	1.98
5	33	3.82	4.00	3.95	33	3.87	4.21	28	1.87	1.50	24	2.19
Mean	30.6	4.06	3.20	4.10	30.4	4.06	3.64	28.1	2.60	2.55	25.4	2.28
MOUSE UNINFECTED												
1	34	5.04	2.38	4.44	34.5	4.35	4.35	35.5	4.18	4.67	36	4.50
2	29.5	2.68	2.70	4.97	30	4.58	3.48	30	3.90	4.75	31	4.45
3	28.5	4.27	4.74	4.98	28	5.50	4.87	29	4.85	5.07	29.5	4.74
Mean	30.7	4.00	3.27	4.80	30.8	4.81	4.23	31.5	4.31	4.83	32.2	4.56

TABLE 2

Oxygen Consumption by Mice Infected with P. berghei and Fed Liquid Synthetic Diet ad libitum, Uninfected Mice Fed Conventional Diet ad libitum and Uninfected Mice Fed Restricted Amounts of Liquid Synthetic Diet Comparable to that Consumed by Infected Mice.

INFECTED, LIQUID DIET

Days After Infection and Starting Diet	8		11		22	
MOUSE	WEIGHT	$cc O_2/g/hr$	WEIGHT	$cc O_2/g/hr$	WEIGHT	$cc O_2/g/hr$
Male 1	29.5	2.42				
Male 2	28	2.12				
Female 3	27	2.53	Died		--	
Female 4	28	2.12				
Mean	28.1	2.30				

UNINFECTED, CONVENTIONAL DIET

Male 1	31	5.36	31	5.47	33	4.85
Male 2	32.5	5.07	32.5	5.54	35	3.54
Mean	31.8	5.22	31.8	5.51	34	4.20

UNINFECTED, RESTRICTED LIQUID DIET

Male 1	30	4.20	27.5	3.96	26	2.94
Male 2	27.5	5.23	25.5	4.20	17	4.23
Female 3	28	4.07	28	4.24	22	4.20
Female 4	25.5	4.55	25	3.99	21	4.28
Mean	27.8	4.51	26.5	4.10	21.5	3.91

red cells. Following phagocytosis the red cell and parasite are digested, but the malaria pigment, hemozoin, remains undigested. Thus, there is an accumulation of Fe-59 in the liver, spleen and bone marrow which is a good measure of phagocytic activity by these organs. Figures 9, 10, 11 and 12 show these differences. There is a significant difference at the 1% level for all values at 48 and 72 hours.

The greater amount of Fe-59 in the liver and spleen of infected mice is not due to increased size. The figures give values as % of infected dose per gram of tissue. The liver of infected mice does not increase significantly (Figure 13) during the 72 hour period of Fe-59 studies, nor does the spleen increase during this time period; however, the spleen is exceedingly increased in size before the 6th day when the Fe-59 experiments started. Although the spleen is enlarged, it continues to function as an organ with phagocytic activity; although not as effectively as the liver.

The data obtained with Fe-59 will be published, and for this reason, the large volume of figures are not included in this report; however, if requested, they will be made available.

Treatment of Mice with pyrimethamine:

Mice treated with pyrimethamine continuously in the diet starting on or before the 6th day of infection would respond with reduction in parasitemia and marked erythropoietic activity. If treatment was started after the 6th day of infection, treatment was frequently unsuccessful. This again suggests the importance of the 6th day of infection.

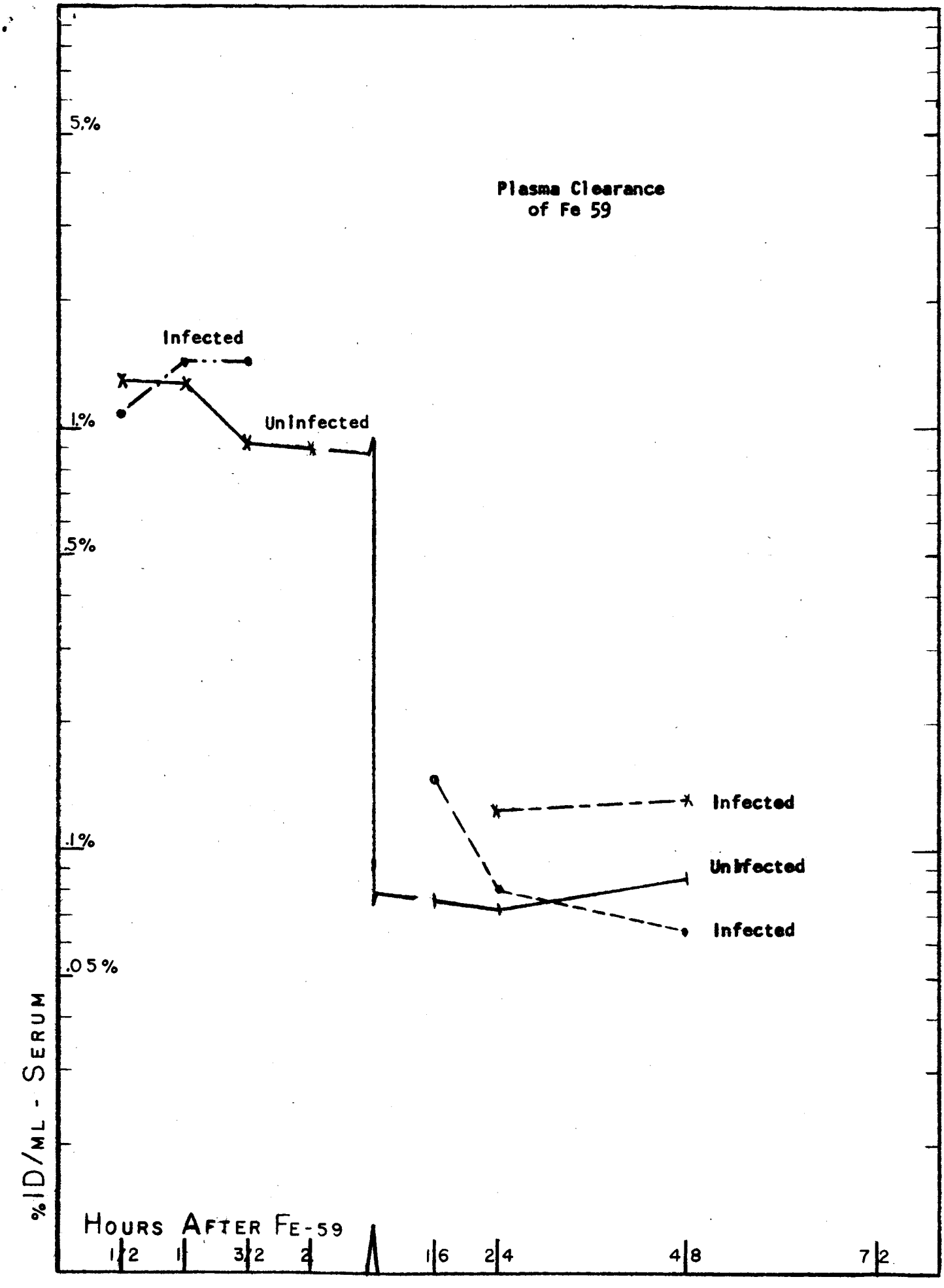
Plasma Clearance
of Fe 59
5%
1%
.5%
.1%
.05%
Infected
Uninfected
Infected
Uninfected
Infected
%ID/ML - SERUM
HOURS AFTER FE-59
1/2
1
3/2
2
1/6
2/4
4/8
7/2

FIGURE 8

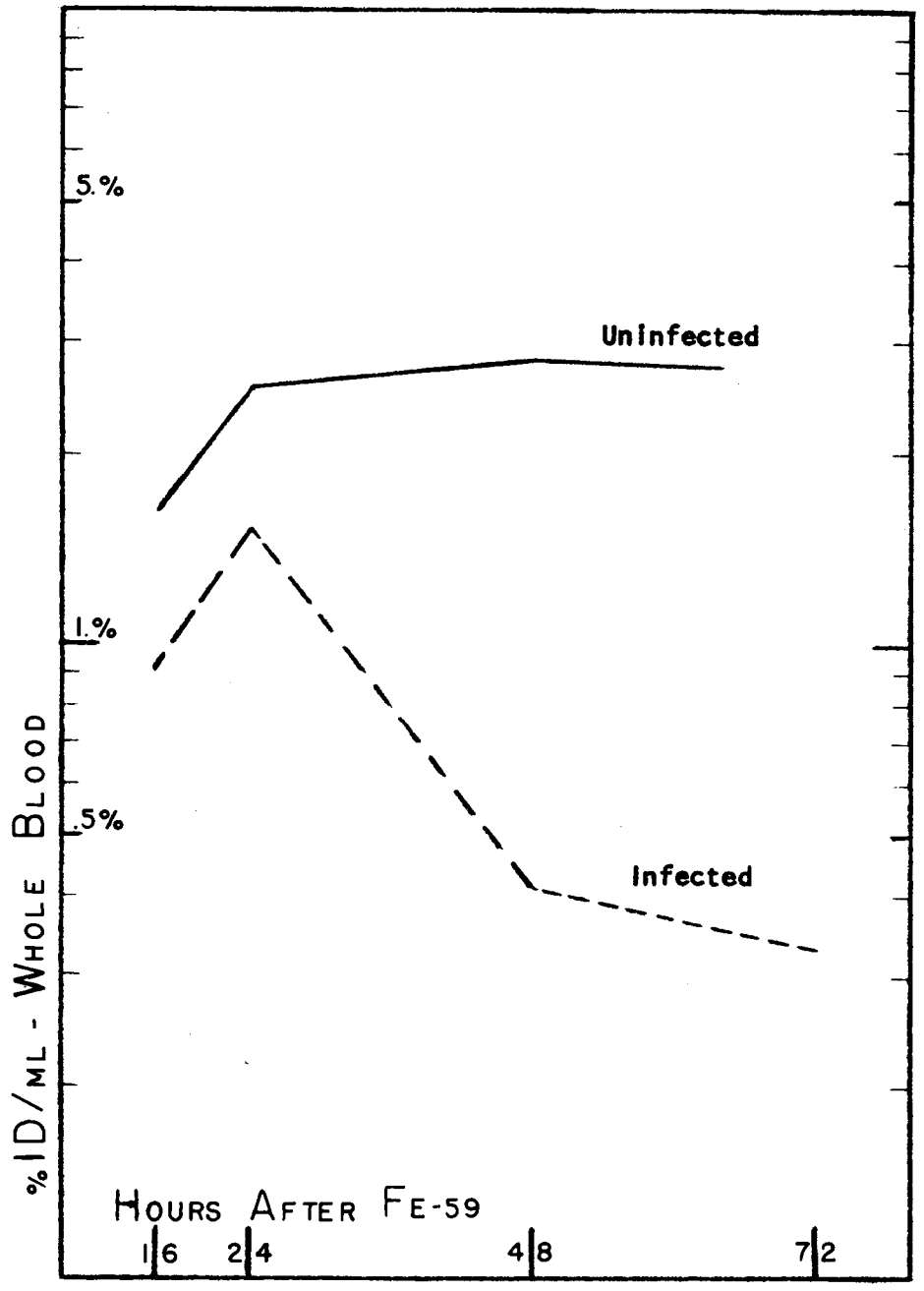
5.%
1.%
.5%
Uninfected
Infected
%ID/ML - Whole Blood
Hours After Fe-59
1
6
2
4
4
8
7
2

FIGURE 9

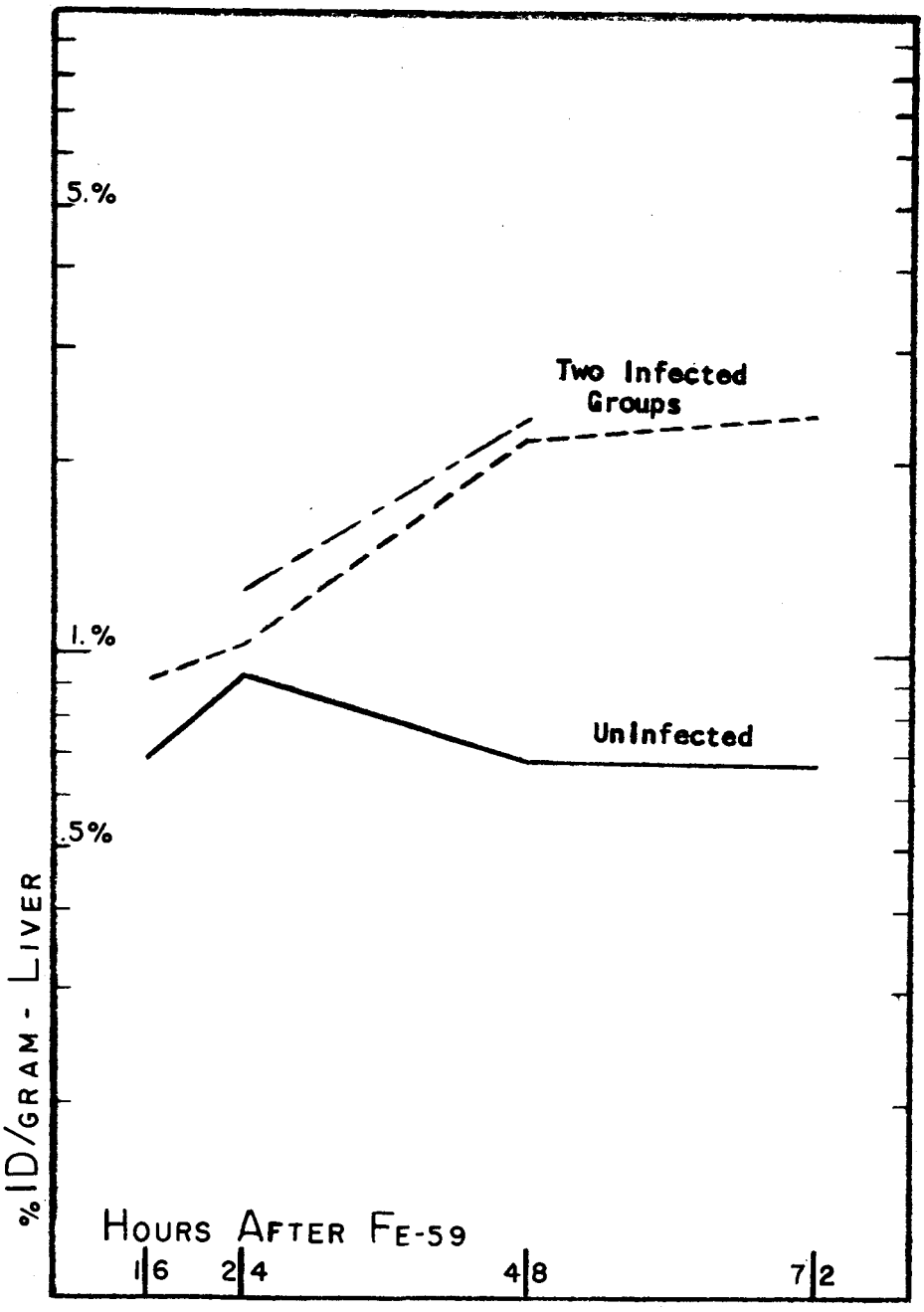
5.%
1.%
.5%
Two Infected Groups
Uninfected
%ID/GRAM - LIVER
HOURS AFTER FE-59
16
24
48
72

FIGURE 10

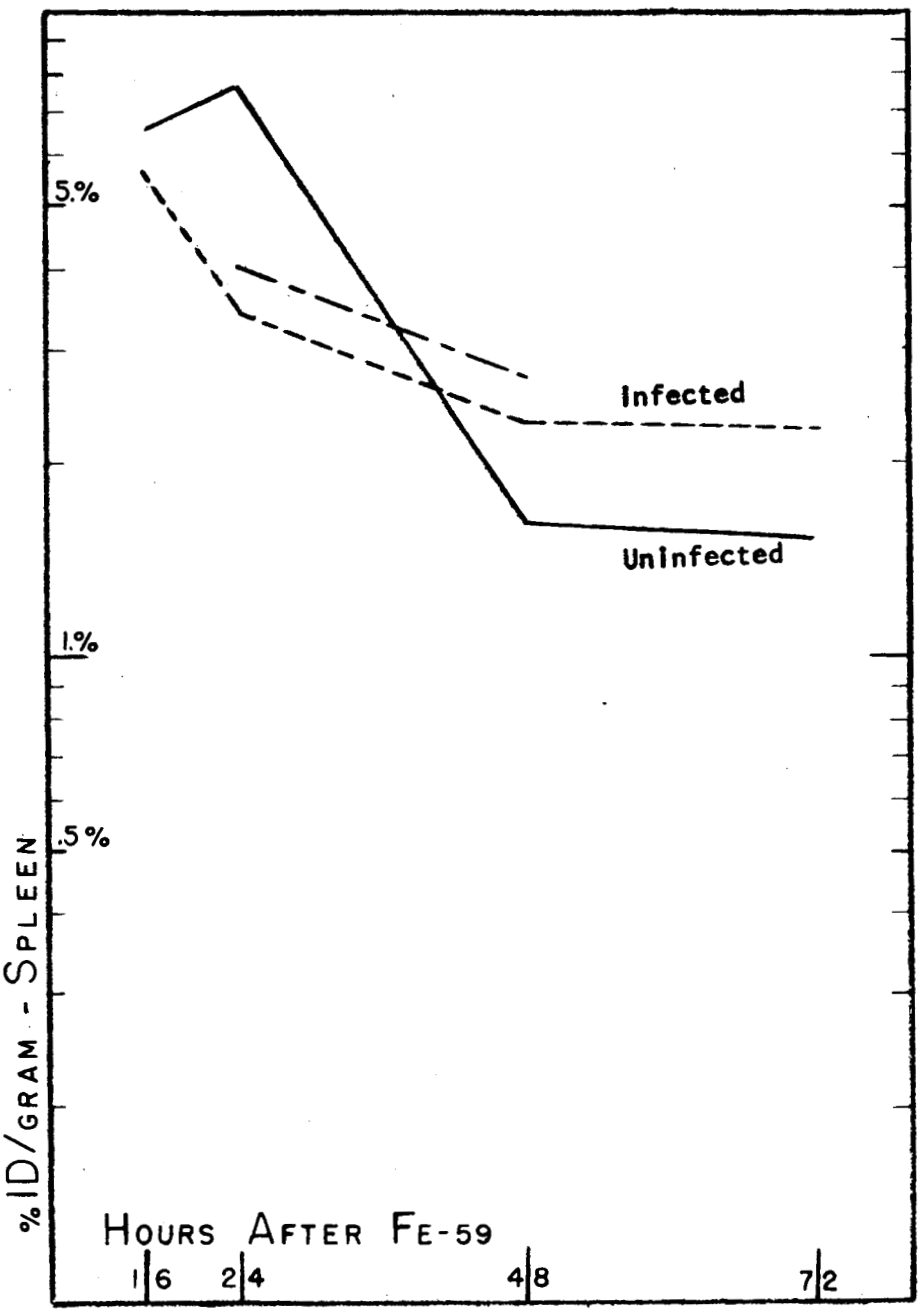
5.%
1.%
.5%
Infected
Uninfected
% ID/GRAM - SPLEEN
HOURS AFTER FE-59
1
6
2
4
4
8
7
2

FIGURE 11

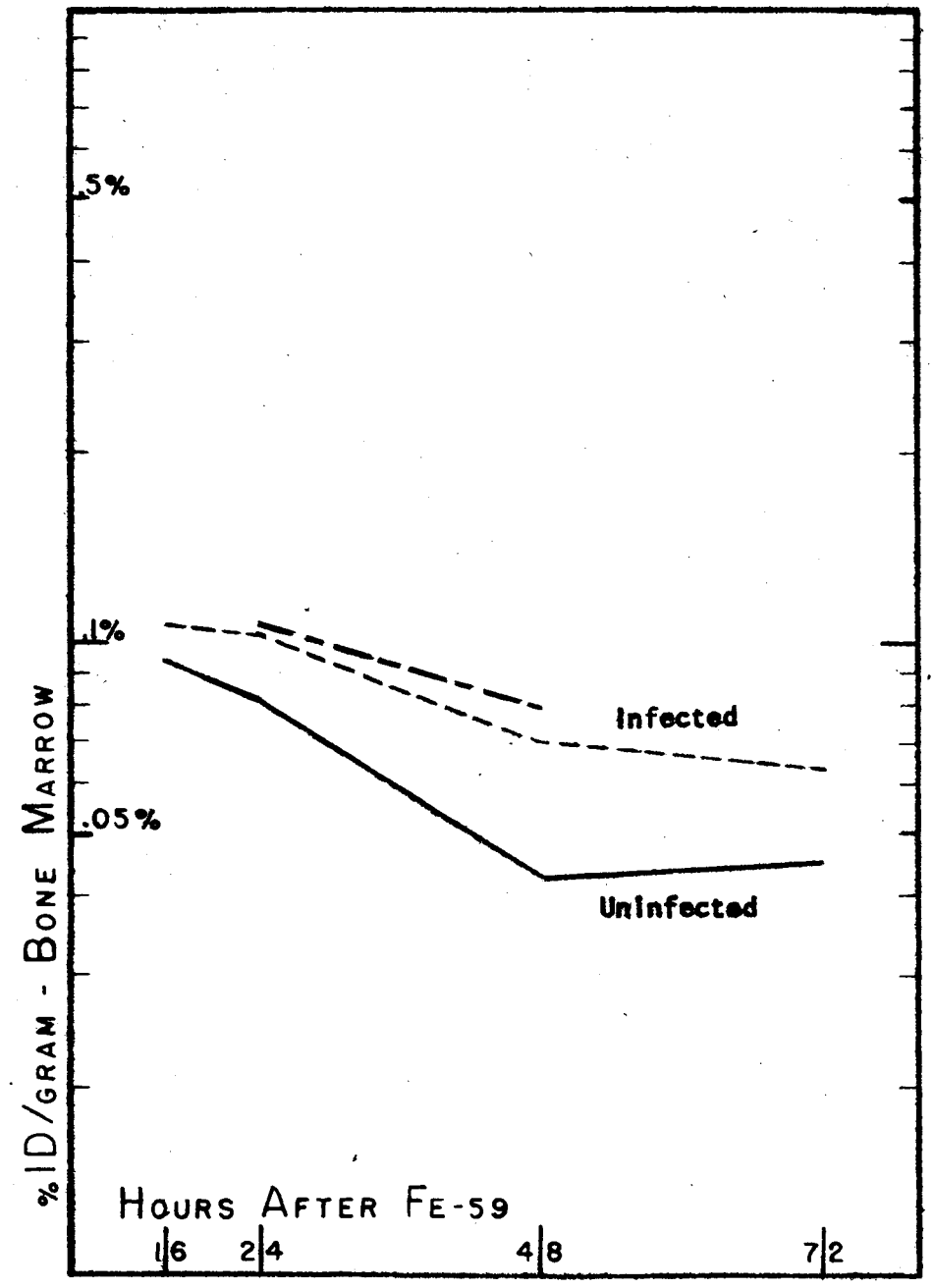
.5%
.1%
.05%
Infected
Uninfected
%ID/GRAM - BONE MARROW
HOURS AFTER FE-59
16
24
48
72

FIGURE 12

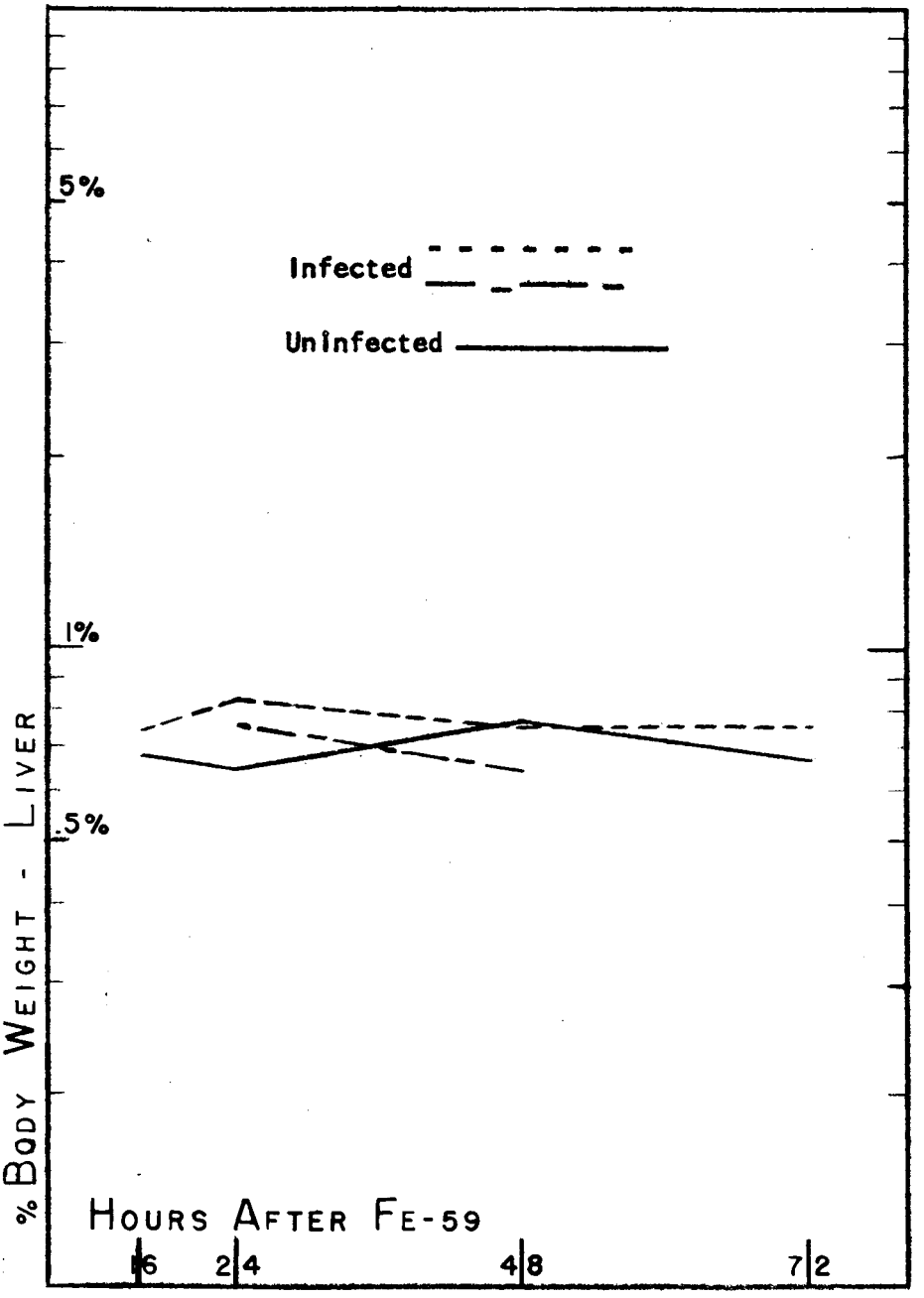
5%
Infected
Uninfected
1%
.5%
% Body Weight - Liver
Hours After Fe-59
16
24
48
72

Figure 13

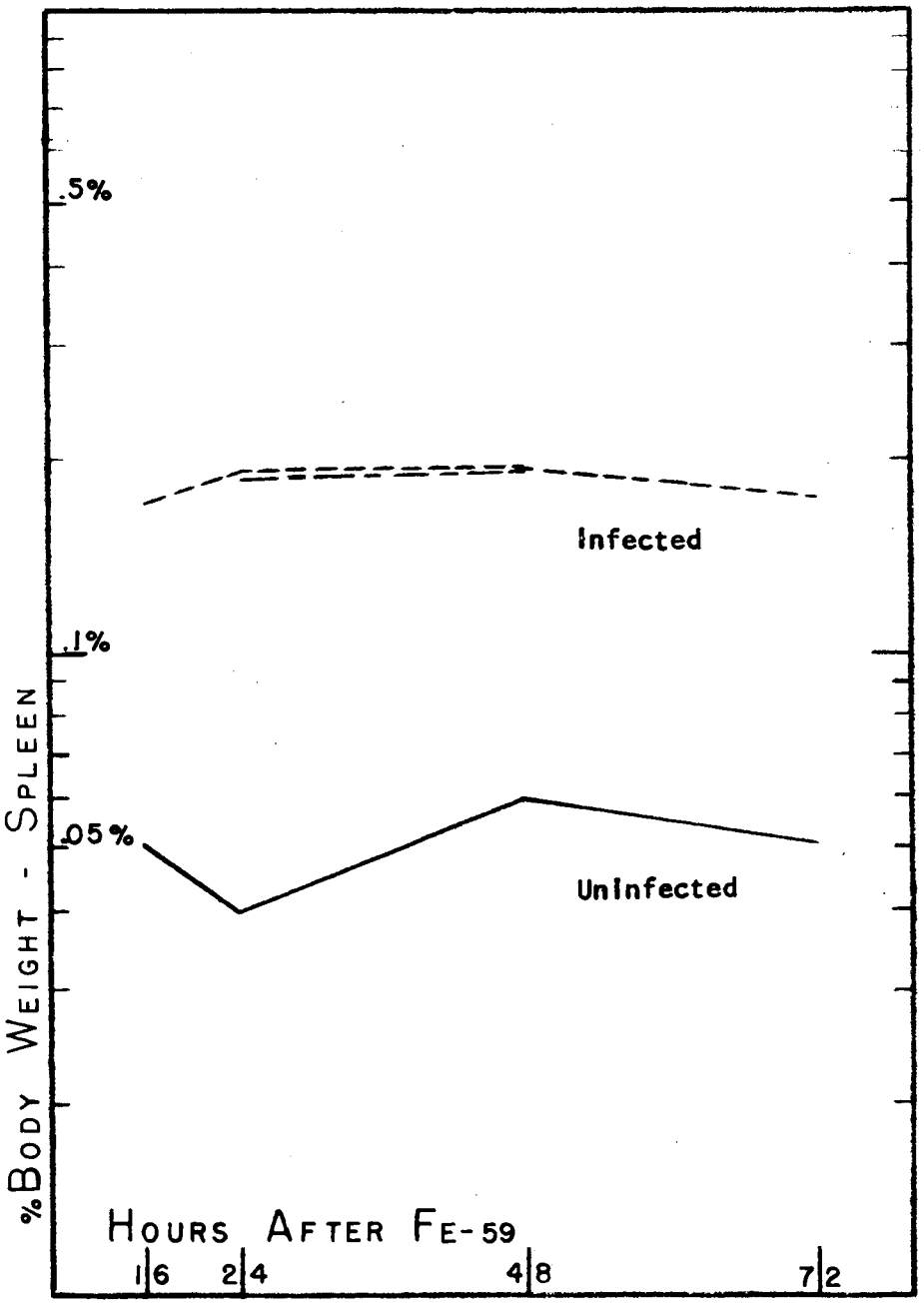
.5%
.1%
.05%
Infected
Uninfected
% Body Weight - Spleen
Hours After Fe-59
16
24
48
72

Figure 14